Arizona Influenza Pandemic Response Plan
Supplement 2: Laboratory Diagnostics

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I. RATIONALE

The goals of diagnostic testing during a pandemic are to:

- Identify the earliest U.S. cases of pandemic influenza (whether the pandemic begins in the United States or elsewhere).
- Support disease surveillance to monitor the pandemic's geographic spread and impact of interventions.
- Facilitate clinical treatment by distinguishing patients with influenza from those with other respiratory illnesses.
- Monitor circulating viruses for antiviral resistance.

During the earliest stages of a pandemic, public health, hospital, and clinical laboratories might receive a large and potentially overwhelming volume of clinical specimens. Pre-pandemic planning is therefore essential to ensure timeliness of diagnostic testing and the availability of diagnostic supplies and reagents, address staffing issues, and disseminate protocols for safe handling and shipping of specimens. Once a pandemic is underway, the need for laboratory confirmation of clinical diagnoses may decrease as the virus becomes widespread.
Diagnostic testing for pandemic influenza virus may involve a range of laboratory assays (see Box 1. and Appendix 1.)

II. OVERVIEW

The public health laboratory is a critical component of the overall public health response to pandemic influenza. The capability of differentiating common influenza from pandemic influenza depends upon the rapid detection and characterization that is available at the Arizona State Public Health Laboratory (ASL) and the Centers for Disease Control and Prevention (CDC).

- The ASL contributes to national laboratory-based surveillance efforts.
- Only through laboratory testing can the signs and symptoms of influenza-like illness be attributed to a definitive pathogen.
- Only by identifying the pathogen can appropriate treatment and control measures be taken to limit/prevent the spread of the disease.
- Once the ASL detects and characterizes a newly emerging influenza strain, for example, the highly pathogenic avian influenza (H5) in the U.S., a sound epidemiologic approach to monitor and respond to the infectious agent can begin.

The ASL plays a key role in laboratory preparedness and response efforts. Federal funding has been used by the ASL not only to enhance biological /chemical terrorism preparedness and response activities but also to improve diagnostic capabilities and capacities for responding to all hazards including pandemic influenza. Specifically the ASL:

- Provides accurate and rapid state-of-the-art testing for detection and identification of newly emergent subtypes of influenza such as H5N1.
- Leads laboratory-based surveillance efforts within each state and contribute to national surveillance efforts as members of a network of World Health Organization collaborating laboratories.

- Provides viral samples to the CDC for further characterization throughout the pandemic period and contribute to the selection of future vaccine strains.

The ASL not only contributes to the detection and identification of influenza, but must also work closely with a network of clinical and physicians office laboratories to support and coordinate diagnostic testing for influenza by:

- Providing education, training, and guidance on use and interpretation of rapid hand-held influenza tests.
- Maintaining a close working relationship with veterinary diagnostic labs to monitor influenza activity within animal populations that may impact human populations.
- Assisting in the development of pandemic preparedness and response plans within states.

III. THE INTERPANDEMIC AND PANDEMIC ALERT PERIODS

Global and US surveillance
The World Health Organization (WHO) has a worldwide network of surveillance laboratories providing information on Influenza. In the United States they work in cooperation with the CDC. The ASL is a participant in the WHO surveillance network (see Box 2.)

Routine surveillance activities
- Once a weekly basis of information about the number of influenza isolates is detected, their sub-types, patient ages, and geographical location are sent to the CDC.
- A random sample of influenza isolates are selected and submitted to the CDC three times during each influenza season. These samples are selected to represent early, middle, and late season isolates.

A. Roles and responsibilities

Clinical and Hospital Laboratories
- Work with the ASL to address laboratory surge capacity issues.
- Train personnel in the management of respiratory specimens during an influenza pandemic.
- Refer specimens from patients with suspected novel influenza to the ASL.
- Institute surveillance for influenza-like illness among laboratory personnel working with influenza virus.

Arizona State Public Health Laboratory
- Performs diagnostic testing.
- Supports surveillance activities
    - Seasonal influenza.
    - Detect novel influenza subtypes.
- Participates in pandemic influenza planning and exercises.
- Institutes surveillance for influenza-like illness among laboratory personnel.
- Develops/reviews pandemic response plans and checklists.

- Educates clinical laboratorians on the safety and handling of specimens suspected to contain novel influenza viruses (see Appendicies 3 and 4).

B. Laboratory testing

Clinical Laboratories

- Test clinical samples by rapid identification methods or viral culture.
- Forward specimens containing suspect novel viruses to the ASL (see page S2-8 for contact information).

Arizona State Public Health Laboratory (ASL)

- All Influenza specimens received by the ASL are tested by Real Time RT-PCR with primer/probe sets for Influenza A (group), Influenza A subtypes H1, H3, H5, and H7, and Influenza B (group).
- PCR positive specimens are inoculated into cell culture for virus isolation
- Hemagglutination Inhibition (HI) testing is done to determine influenza A subtype or influenza B subtype.
- Refers specimens to the CDC if a patient meets the requirements for infection with a novel influenza virus and tests positive for Influenza A Virus.

C. Laboratory safety - biocontainment

During the Pandemic Alert Period, specimens from suspected cases of human infection with novel influenza viruses should be sent to the ASL for testing. The following guidelines should be used for handling and testing of samples suspected to contain a novel influenza virus.

- Commercial antigen detection testing – conduct all assays in a Bio-Safety cabinet under BSL-II conditions.
- RT-PCR – conduct all assays in a Bio-Safety cabinet under BSL-II conditions.
- Virus Isolation - all assays must be conducted under BSL-III with enhancements. (see Appendix 4 for additional laboratory BioSafety Guidelines).

D. Surge capacity planning

1. Staffing and Training

- Cross-train personnel in the use of testing protocols and reporting through existing surveillance systems.
- Establish back-up plans for hiring temporary laboratory staff.

2. Supplies and Equipment

- Establish inventory system to determine current level of diagnostic supplies, including personal protective equipment.
- Determine mechanism to monitor consumption of supplies during the pandemic.
- Assess anticipated equipment and supply needs.

E. Partnerships

The ASL should build partnerships with the private clinical laboratories and provide them with updated information and training in influenza diagnostics.

IV. THE PANDEMIC PERIOD

A. Roles and responsibilities

Public health, hospital and clinical laboratories will continue to support surveillance for pandemic influenza through the same mechanisms that support laboratory-based surveillance for seasonal influenza.

Clinical Laboratories

- Perform diagnostic testing for influenza.
- Scale up to manage increased numbers of requests for influenza testing.
- Support surveillance activities – refer selected specimens from possible pandemic influenza patients to the ASL.
- Maintain other diagnostic services.

Arizona State Public Health Laboratory (ASL)

- Maintain surveillance activities.
- Scale up to manage increased numbers of requests for influenza testing.
- Work with federal partners to supply healthcare providers and clinical laboratories with guidelines on all aspects of specimen management and diagnostic testing.
- Work with federal partners to monitor the pandemic virus and conduct special studies with CDC related to vaccine development or other aspects of emergency response.
- Maintain reference testing for influenza.
- Continue education of clinicians & laboratorians.
- Share data/information in "real-time".
- Maintain other diagnostic services.

B. Laboratory support for healthcare providers

Arizona State Public Health Laboratory (ASL)

- Provide clinical laboratories with guidelines for safe handling, processing, and rapid diagnostic testing of clinical specimens from patients who meet the case definition for pandemic influenza (see Appendices 4 and 5).
- Provide rapid communication of test results.
- Provide guidance on the use of commercially available rapid diagnostic tests for the detection of Influenza A (see Appendix 6).
- Provide guidance on the specimens to refer to the State Public Health Laboratory.

C. Laboratory safety - biocontainment

- Commercial antigen detection testing – conduct all assays in a Bio-Safety cabinet under BSL-II conditions.
- RT-PCR – conduct all assays in a Bio-Safety cabinet under BSL-II conditions.
- Virus Isolation - all assays must be conducted under Bio-Safety Level III with enhancements. (see Appendix 4).

D. Occupational health issues for laboratory workers

To protect the health of laboratory workers during a pandemic, laboratories should maintain the safety practices used during the Interpandemic and Pandemic Alert Periods.

- Conduct laboratory procedures under appropriate biocontainment conditions.
- Encourage routine vaccination of laboratory employees exposed to specimens with respiratory infections. (see Appendix 7).

E. Use of diagnostic assays during an influenza pandemic

1. Rapid Diagnostic Tests

Rapid diagnostic tests based on antigen detection are commercially available for influenza. Laboratories in outpatient settings and hospitals can use these tests to detect viruses in 30 minutes. Some tests can detect Influenza A viruses, including avian strains. Testing is not capable of distinguishing between the subtypes of influenza.
(see Appendix 6).

2. RT-PCR Subtyping

Influenza specimens may be typed and subtyped using RT-PCR. This method does not require the growth or isolation of virus.

3. Virus Isolation

This method requires growth of virus in cell culture. Identification of the virus is usually confirmed through the use of IFA staining or hemagglutination inhibition (HAI), or RT-PCR to monitor circulating seasonal strains. If clinical or epidemiological data suggests that the human case of influenza might be due to infection with avian influenza, the virus should not be cultured except under BSL-3 conditions with enhancements. Laboratories that lack BSL-3 enhanced facilities should contact their State Public Health Laboratory and arrange to forward the specimen to the CDC for isolation and characterization.

4. Immunofluorescence Antibody Staining

IFA staining following virus isolation may be used by some laboratories to identify influenza types (A & B) and Influenza A subtypes using a panel of specific antisera.

5. Serologic Tests

Tests based on the detection of antibodies in the patient's sera can be used retrospectively to confirm influenza detection. Acute and convalescent (paired) sera are used to detect rising antibody titers in patient's sera. The testing cannot be used to subtype species of Influenza A Virus. This method is of limited value in the monitoring of an ongoing influenza pandemic.

V. APPENDICES

Reference Testing Guidelines

The ASL and other local laboratories may conduct initial testing on patient specimens for influenza A or potential highly pathogenic strains, if laboratory capacity is available. Due to the spread of avian influenza A (H5N1) in poultry in Asia, laboratories should be on the alert for avian and human H5 viruses. Procedures for diagnosis of human cases of influenza A (H5N1) are provided in Appendix 2. Influenza A viruses other than currently circulating H1 and H3 subtypes should also be considered as potentially pandemic if detected in humans.
(see Appendix 3).

Box 1. Use of diagnostic assays during an influenza pandemic

Public health and clinical laboratories will use different types of diagnostic tests for influenza at different stages of a pandemic. Each of the tests discussed below is described in detail in Appendix 1.

Virus Isolation
Virus isolation—growing the viral strain in cell culture—is the "gold standard" for influenza diagnostics because it confirms that the virus is infectious. During a pandemic, virus isolation followed by antigenic and genetic (sequencing) analysis will be used to characterize the earliest pandemic isolates, as well as to monitor their evolution during the pandemic. Laboratories that participate in the WHO Global Influenza Surveillance Network, such as ASL, typically use virus isolation followed by hemagglutination inhibition (HAI), IFA staining, or RT-PCR to monitor circulating seasonal strains of influenza. If clinical and epidemiologic data suggest that a human case of influenza might be due to infection with avian influenza A (H5N1) or another highly pathogenic avian influenza strain (see Box 3), the virus should not be cultured except under BSL-3 conditions with enhancements. Laboratories that lack BSL-3 enhanced facilities may either perform
RT-PCR subtyping using BSL-2 containment procedures or send the specimen to CDC for isolation and characterization.

Immunofluorescence Antibody Staining
IFA staining following virus isolation can be used to identify influenza types (A, B) and influenza A HA subtypes using a panel of specific antisera. In some cases, IFA can be used for direct testing of cells pelleted from original clinical samples. CDC's Influenza Branch produces and distributes a reagent kit to WHO collaborating laboratories that includes monoclonal antibodies for typing and subtyping currently circulating influenza viruses by IFA. Many laboratories use commercially available reagents to type influenza viruses by direct immunofluorescence tests (DFA).
Box 1. Use of diagnostic assays during an influenza pandemic – cont.

RT-PCR Subtyping
Influenza specimens may also be typed and subtyped using RT-PCR, which does not require *in vitro* growth or isolation of virus. ASL scientists have received training from CDC on using RT-PCR subtyping to identify human and avian HA subtypes of public health concern. APHL members can access protocols and sequences of primers and probes that can be used for typing and subtyping on the APHL website.

Serologic Tests
Tests based on detection of antibodies in patient sera—e.g., enzyme-linked immunosorbent assay (ELISA), HAI, and microneutralization assay—can be used to retrospectively confirm influenza infection. Although microneutralization assay is the most comprehensive test for detection in humans of antibodies to avian influenza viruses, it is currently not available at ASL.

Box 1. Use of diagnostic assays during an influenza pandemic – cont.

Rapid Diagnostic Tests

Several rapid diagnostic test kits based on antigen detection are commercially available for influenza. Laboratories in outpatient settings and hospitals can use these tests to detect influenza viruses within 30 minutes. Some tests can detect influenza A viruses (including avian strains); others can detect influenza A and B viruses without distinguishing between them, and some can distinguish between influenza A and B viruses. The type of specimens used in these tests (i.e., nasal wash/aspirate, nasopharyngeal swabs, or nasal swab or throat swab) may also vary. Like RT-PCR, rapid diagnostic tests do not require *in vitro* growth or isolation of virus. During a pandemic, rapid diagnostic tests will be widely used to distinguish influenza A from other respiratory illnesses. See Appendix 6 for additional information.

Box 2. Laboratory support for seasonal influenza surveillance

U.S. Collaborating Laboratories of the WHO Global Influenza Surveillance Network
All state public health laboratories, including ASL, as well as about 25 tertiary-care hospital and academic center laboratories, participate as U.S. collaborating laboratories in the WHO Global Influenza Surveillance Network, which collects worldwide data on circulating strains of influenza viruses. These data are used to develop recommendations for the formulation of each year's influenza vaccines, as well as to detect new human influenza viruses that might have pandemic potential. CDC's Influenza Laboratory serves as the WHO Collaborating Center for Surveillance, Epidemiology, and Control of Influenza, along with the WHO Collaborating Centers for Reference and Research on Influenza in Australia, Japan, and the United Kingdom. The U.S.-based WHO collaborating laboratories provide CDC with weekly reports of laboratory-confirmed cases of influenza A and B viruses, by age group. These laboratories typically use virus isolation followed by antigenic testing with IFA staining or HAI—or by molecular testing with RT-PCR—to identify known subtypes of human influenza viruses. If unusual subtypes are detected, or if the specimens cannot be subtyped using available techniques, the specimens are sent to CDC for further testing.

NREVSS Collaborating Laboratories
The National Respiratory and Enteric Virus Surveillance System (NREVSS; http://www.cdc.gov/ncidod/dvrd/revb/nrevss/) includes more than 90 laboratories throughout the country, including many hospital laboratories, some state public health laboratories, and a few private commercial laboratories. About 40 of the NERVSS laboratories are also WHO collaborating laboratories. Like the WHO collaborating laboratories, NREVSS laboratories provide CDC with weekly reports of laboratory confirmed cases of influenza A and B viruses. These laboratories typically test respiratory specimens with commercially available rapid diagnostic tests. Several NREVSS laboratories also perform virus isolation followed by rapid diagnostic tests or antigenic typing by IFA. If untypable viruses or unusual subtypes are detected, the specimens are sent to the state public health laboratory or to CDC for further testing.

Box 3. Avian influenza strains with high and low pathogenicity

The U.S. Department of Agriculture (USDA) classifies avian influenza viruses as low pathogenic avian influenza (LPAI) viruses or highly pathogenic avian influenza (HPAI) viruses, based on characteristics of a virus' hemagglutinin cleavagesite or its virulence in birds, as determined by laboratory testing. LPAI strains are endemic in wild birds worldwide and are responsible for most avian influenza outbreaks in poultry. LPAI strains with H5 and H7 subtypes sometimes evolve into highly pathogenic forms. HPAI strains are extremely contagious and cause severe illness and high mortality rates in poultry.

LPAI strains include:
•H5N2, the cause of poultry outbreaks in New York, Maine, and California in 2002
•H7N2, the cause of poultry outbreaks in Delaware, Maryland, and New Jersey in 2004

HPAI strains include:
•H5N1, the cause of major poultry outbreaks in Southeast Asia
•H7N7, the cause of a 2003 outbreak in the Netherlands
•H7N3, the cause of a 2004 outbreak in British Columbia
•H5N2, the cause of a 2004 outbreak in poultry in Texas

The 2004 outbreak in Texas was the first HPAI outbreak in the United States since a previous outbreak of H5N2 in1983-84 in the northeastern United States. The 1983-84 disease control effort involved the destruction of approximately 17 million birds and cost more than $70 million.

Although avian influenza A viruses do not usually infect humans, several instances of human infections of avian influenza have been reported since 1997. Cases of avian influenza infection in humans are apparently caused by contact with infected poultry or with surfaces contaminated with avian influenza viruses.

LPAI strains associated with human infection include:
•H9N2, which caused three cases of influenza-like illness in Hong Kong between 1999 and 2003, and other casesin China in 1998 and 1999
•H7N2, which was detected by serology in one person involved in the culling of sick chickens during the responseto a poultry outbreak in Virginia in 2002, and was isolated from a New York resident in 2003 (unknown source of the infection)

HPAI viruses associated with human infection include:
•H5N1, which caused 51 deaths in Southeast Asia between January 2004 and April 2005•H7N7, which caused the death of a veterinarian as well as 83 cases of mild human disease (including conjunctivitis) during the 2003 poultry outbreak in the Netherlands.
•H7N3, which caused 2 cases of very mild human disease (conjunctivitis, headache) in persons culling sick poultry in British Columbia in 2004

Appendix 1.
Influenza diagnostic assays

Among the several types of assays used to detect influenza, rapid antigen tests, reverse-transcription polymerase chain reaction (RT-PCR), viral isolation, immunofluorescence assays (IFA), and serology are the most commonly used. The sensitivity and specificity of any test for influenza will vary by the laboratory that performs the test, the type of test used, and the type of specimen tested. A chart that lists influenza diagnostic procedures and commercially available rapid diagnostic tests follows more detailed descriptions provided below.

Virus Isolation
Biocontainment level: Interpandemic and Pandemic Alert Periods – BSL-3 with enhancements; Pandemic Period –BSL-2

Virus isolation is a highly sensitive and very useful technique when the clinical specimens are of good quality and have been collected in a timely manner (optimally within 3 days of the start of illness). Isolation of a virus in cell culture along with the subsequent identification of the virus by immunologic or genetic techniques are standard methods for virus diagnosis. Virus isolation amplifies the amount of virus from the original specimen, making a sufficient quantity of virus available for further antigenic and genetic characterization and for drug-susceptibility testing if required. Virus isolation is considered the "goldstandard" for diagnosis of influenza virus infections.

Highly pathogenic avian influenza (HPAI) viruses are BSL-3 agents. During the Interpandemic and Pandemic Alert Periods, laboratories should attempt to culture HPAI viruses—as well as other influenza viruses with pandemic potential—only under BSL-3 conditions with enhancements in order to optimally reduce the risk of a novel influenza virus subtype spreading to persons or animals. During the Pandemic Period, biocontainment of BSL-2 is appropriate to prevent laboratory-acquired infection and the virus will already be widespread.

In recent years, the use of cell lines has surpassed the use of embryonated eggs for culturing of influenza viruses, although only viruses grown in embryonated eggs are used as seed viruses for vaccine production. Because standard isolation procedures require several days to yield results, they should be used in combination with the spin-amplification shell-vial method. The results of these assays can be obtained in 24–72 hours, compared to an average of 4.5 days using standard culture techniques. Spin-amplification should not be performed using 24-well plates because of increased risk of cross-contamination. The most effective combination of cell lines recommended for public health laboratories is primary rhesus monkey for standard culture, along with Madin Darby Canine Kidney (MDCK) in shell vial. [1]The use of these two cell lines in combination has demonstrated maximum sensitivity over time for recovery of evolving influenza strains. Some clinical laboratories have recently reported good isolation rates using commercially available cell-line mixed-cell combinations; however, data are lacking on the performance of these mixed cells with new subtypes of Influenza A viruses.

[1] The shell-vial technique is described in: *Manual of Clinical Virology*, 3rd edition. Steven Specter, Richard Hodinka, and Stephen Young, eds. ASM Press, 2000.

Appropriate clinical specimens for virus isolation include nasal washes, nasopharyngeal aspirates, nasopharyngeal and throat swabs, tracheal aspirates, and bronchoalveolar lavage. Ideally, specimens should be collected within 72 hours of the onset of illness. Viral culture isolates are used to provide specific information regarding circulating influenza subtypes and strains. This information is needed to compare current circulating influenza strains with vaccine strains, to guide decisions on influenza treatment and chemoprophylaxis, and to select vaccine strains for the coming year. Virus isolates also are needed to monitor the emergence of antiviral resistance and of novel influenza A subtypes that might pose a pandemic threat. During outbreaks of influenza-like illness, viral culture may help identify other causes of illness when influenza is not the etiology (except when using MDCK cells or the MDCK shell-vial technique).

Immunofluorescence Assays
Biocontainment level: BSL-2 when performed directly on clinical specimens; if used on cultures for earlier detection of virus, biocontainment recommendations for viral culture apply

Direct (DFA) or indirect (IFA) immunofluorescence antibody staining of virus-infected cells is a rapid and sensitive method for diagnosis of influenza and other viral infections. DFA and IFA can also be used to type and subtype influenza viruses using commercially available monoclonal antibodies specific for the influenza virus HA. The sensitivity of these methods is greatly influenced by the quality of the isolate, the specificity of the reagents used, and the experience of the person(s) performing, reading, and interpreting the test.

Although IFA can be used to stain smears of clinical specimens directly, when rapid diagnosis is needed it is preferable to first increase the amount of virus through growth in cell culture. For HPAI isolates, attempts to culture the virus should be made only under BSL-3 conditions with enhancements.

Reverse-Transcription Polymerase Chain Reaction (RT-PCR)
Biocontainment level: BSL-2

PCR can be used for rapid detection and subtyping of influenza viruses in respiratory specimens. Because the influenza genome consists of single-stranded RNA, a complementary DNA (cDNA) copy of the viral RNA must be synthesized using the reverse-transcriptase (RT) enzyme prior to the PCR reaction.

APHL member laboratories can obtain CDC protocols and sequences of primers and probes for rapid RT-PCR detection of human and avian HA subtypes of current concern at the APHL website (ASL is an APHL member laboratory and has these capabilities) . These protocols use real-time RT-PCR methods with fluorescent-labeled primers that allow automatic, semi-quantitative estimation of the input template. The RT-PCR results are analyzed and archived electronically, without the need for gel electrophoresis and photographic recording. A large number of samples may be analyzed at the same time, reducing the risk of carry-over contamination. As with all PCR assays, interpretation of real-time RT-PCR tests must account for the possibility of false-negative and false-positive results. False-negative results can arise from poor sample collection or degradation of the viral RNA during shipping or storage.

Application of appropriate assay controls that identify poor-quality samples (e.g., an extraction control and, if possible, an inhibition control) can help avoid most false-negative results.[2] The most common cause of false-positive results is contamination with previously amplified DNA. The use of real-time RT-PCR helps mitigate this problem by operating as a contained system. A more difficult problem is the cross-contamination that can occur between specimens during collection, shipping, and aliquoting in the laboratory. Use of multiple negative control samples in each assay and a well-designed plan for confirmatory testing can help ensure that laboratory contamination is detected and that negative specimens are not inappropriately identified as influenza-positive.

Specimens that test positive for a novel subtype of influenza virus should be forwarded to CDC for confirmatory testing. (Due to the possibility of contamination, it is important to provide original clinical material.) All laboratory results should be interpreted in the context of the clinical and epidemiologic information available on the patient.

Rapid Diagnostic Tests
Biocontainment level: BSL-2

Commercial rapid diagnostic tests can be used in outpatient settings to detect influenza viruses within 30 minutes. These rapid tests differ in the types of influenza viruses they can detect and in their ability to distinguish among influenza types. Different tests can 1) detect influenza A viruses only (including avian strains); 2) detect both influenza A and B viruses, without distinguishing between them; or 3) detect both influenza A and B viruses and distinguish between them.

The types of specimens acceptable for use (i.e., nasal wash/aspirate, nasopharyngeal swab, or nasal swab and throat swab) also vary by test. The specificity and, in particular, the sensitivity of rapid tests are lower than for viral culture and vary by test and specimen tested. The majority of rapid tests are >70% sensitive and >90% specific. Thus, as many as 30% of samples that would be positive for influenza by viral culture may give a negative rapid test result with these assays. When interpreting results of a rapid influenza test, physicians should consider the level of influenza activity in the community. When influenza prevalence is low, positive rapid test results should be independently confirmed by culture or RT-PCR. When influenza is known to be circulating, clinicians should consider confirming negative tests with viral culture or other means because of the lower sensitivity of the rapid tests. Package inserts and the laboratory performing the test should be consulted for more details regarding use of rapid diagnostic tests. Additional information on diagnostic testing is provided at: http://www.cdc.gov/flu/professionals/labdiagnosis.htm . Detailed information on the use of rapid diagnostics tests is provided in Appendix 6.

[2] CDC is working with the private sector to provide inactivated RNA virus for use as RT-PCR controls for influenza A (H5) testing in LRN laboratories. CDC is working with USDA to resolve any permit issues that might affect the ability of LRN members to use these controls.

Serologic Tests[3]

Hemagglutination Inhibition (HAI)
Biocontainment level: BSL-2

Serologic testing can be used to identify recent infections with influenza viruses. It can be used when the direct identification of influenza viruses is not feasible or possible (e.g., because clinical specimens for virus isolation cannot be obtained, cases are identified after shedding of virus has stopped, or the laboratory does not have the resources or staff to perform virus isolation).

Since most human sera contain antibodies to influenza viruses, serologic diagnosis requires demonstration of a four-fold or greater rise in antibody titer using paired acute and convalescent serum samples. HAI is the preferred diagnostic test for determining antibody rises. In general, acute-phase sera should be collected within one week of illness onset, and convalescent sera should be collected 2–3 weeks later.

There are two exceptions in which the collection of single serum samples can be helpful in the diagnosis of influenza. In investigations of outbreaks due to novel viruses, testing of single serum samples has been used to identify antibody to the novel virus. In other outbreak investigations, antibody test results from single specimens collected from persons in the convalescent phase of illness have been compared with results either from age-matched persons in the acute phase of illness or from non-ill controls. In such situations, the geometric mean titers between the two groups to a single influenza virus type or subtype can be compared. In general, these approaches are not optimal, and paired sera should be collected whenever possible.

Because HAI titers of antibodies in humans infected with avian influenza viruses are usually very low or even undetectable, more sensitive serologic tests, such as microneutralization, may be needed.

Microneutralization Assay
Biocontainment level: Interpandemic and Pandemic Alert Periods – BSL-3 with enhancements; Pandemic Period –BSL-2

The virus neutralization test is a highly sensitive and specific assay for detecting virus-specific antibody in animals and humans. The neutralization test is performed in two steps: 1) a virus-antibody reaction step, in which the virus is mixed with antibody reagents, and 2) an inoculation step, in which the mixture is inoculated into a host system (e.g. cell cultures,embryonated eggs, or animals). The absence of infectivity constitutes a positive neutralization reaction and indicates the presence of virus-specific antibodies in human or animal sera. The virus neutralization test gives the most precise answer to the question of whether or not a person has antibodies that can neutralize the infectivity of a given virus strain. The neutralization test has several additional advantages for detecting antibody to influenza virus. First, the assay primarily detects antibodies

[3]Enzyme-linked immunoassay (EIA) is not included on this list because of non-specificity issues. Complement fixation is not included because it is currently out of use.

to the influenza virus HA and thus can identify functional, strain-specific antibodies in animal and human serum. Second, since infectious virus is used, the assay can be developed quickly upon recognition of a novel virus and before suitable purified viral proteins become available for use in other assays.

The microneutralization test is a sensitive and specific assay for detecting virus-specific antibody to avian influenza A (H5N1)in human serum and potentially for detecting antibody to other avian subtypes. Microneutralization can detect H5-specificantibody in human serum at titers that cannot be detected by HAI. Because antibody to avian influenza subtypes is presumably low or absent in most human populations, single serum samples can be used to screen for the prevalence of antibody to avian viruses. However, if infection of humans with avian viruses is suspected, the testing of paired acute and convalescent sera in the microneutralization test would provide a more definitive answer regarding the occurrence of infection. Conventional neutralization tests for influenza viruses based on the inhibition of cytopathogenic effect (CPE)-formation in MDCK cell cultures are laborious and rather slow, but in combination with rapid culture assay principles the neutralization test can yield results within 2 days. For HPAI viruses, neutralization tests should be performed at BSL-3 enhanced conditions.

QUICK REFERENCE CHART OF INFLUENZA DIAGNOSTIC TESTS[1]

(From: *Prevention and Control of Influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP)*. MMWR 2004;53(RR-6):1-40.

Procedure	Influenza Types Detected	Acceptable Specimens	Time for Results	Rapid result available
Viral culture	A and B	nasal wash/aspirate, NP swab,2 nasal aspirate, nasal swab and throat swab, sputum	5–10 days[3]	No
Immunofluorescence Antibody Staining	A and B	nasal wash/aspirate, NP swab,2 nasal aspirate, nasal swab and throat swab, sputum	2–4 hours	No
RT-PCR[5]	A and B	nasal wash/aspirate, NP swab,2 nasal aspirate, throat swab, bronchial wash, nasal aspirate, sputum	Hours	No
Serology	A and B	paired acute/convalescent serum samples6	>2 weeks	No
Rapid Diagnostic Tests				
Directigen Flu A[7] (Becton-Dickinson)	A	NP swab,2 throat swab, nasal wash, nasal aspirate	See insert	Yes
Directigen Flu A+B[7,9] (Becton-Dickinson)	A and B	NP swab,2 throat swab, nasal wash, nasal aspirate	See insert	Yes
FLU OIA[7] (Thermo Electron)	A and B[4]	NP swab,2 throat swab, nasal aspirate, sputum	See insert	Yes
FLU OIA A/B[7,8] (Thermo Electron)	A and B	NP swab,2 throat swab, nasal aspirate, sputum	See insert	Yes
XPECT Flu A/B[7,8] (Remel)	A and B	Nasal wash, NP swab,2 throat swab	See insert	Yes
NOW Flu A Test[7,8] NOW Flu B Test[7,9] (Binax)	A B	Nasal wash, NP swab2 Nasal wash, NP swab2	See insert	Yes Yes
QuickVue Influenza Test[8] (Quidel)	A and B[4]	NP swab,2 nasal wash, nasal aspirate	See insert	Yes
QuickVue Influenza A+B Test[8] (Quidel)	A and B[9] A	NP swab,2 nasal wash, nasal aspirate	See insert	Yes
SAS Influenza A[7,9] SAS Influenza B[7,8]	B	NP wash,2 NP aspirate2 NP wash,2 NP aspirate2	See insert	Yes Yes
ZstatFlu[8] (ZymeTx)	A and B[4]	throat swab	See insert	Yes

[1] The list might not include all FDA-approved test kits.
[2] NP = nasopharyngeal
[3] Shell-vial culture, if available, may reduce time for results to 2 days.
[4] Does not distinguish between influenza A and B virus infections.
[5] RT-PCR = reverse-transcription polymerase chain reaction
[6] A fourfold or greater rise in antibody titer from the acute- (collected within the first week of illness) to the convalescent-phase sample (collected 2–4 weeks after the acute sample) indicates recent infection.
[7] Moderately complex test that requires specific laboratory certification
[8] CLIA-waived test. Can be used in any office setting. Requires a certificate of waiver or higher laboratory certification
[9] Distinguishes between influenza A and B virus infections.

Disclaimer: Use of trade names or commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or the Department of Health and Human Services

Appendix 2.
Interim CDC recommendations: enhanced U.S. surveillance and diagnostic evaluation to identify cases of human infection with avian influenza a (H5N1)

NOTE: This guidance pertains to the avian influenza A (H5N1) situation in October 2005. CDC will provide updated guidance for avian influenza A (H5N1) and for new situations, as needed, through the Health Alert Network (HAN).

Enhanced surveillance efforts by state and local health departments, hospitals, and clinicians are needed to identify patients at increased risk for influenza A (H5N1). Interim recommendations include the following:

Testing for avian influenza A (H5N1) is indicated for hospitalized patients with:

•Radiographically confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternative diagnosis has not been established, *and*
•History of travel within 10 days of symptom onset to a country with documented avian influenza A (H5N1) infections in poultry and/or humans. (For a regularly updated listing of H5N1-affected countries, see the OIE website at http://www.oie.int/eng/en_index.htm and the WHO website at http://www.who.int/en/).

or

Testing for avian influenza A (H5N1) should be considered on a case-by-case basis in consultation with state and local health departments for hospitalized or ambulatory patients with:

- Documented temperature of >100.4°F (>38°C), *and*
- One or more of the following: cough, sore throat, or shortness of breath, *and*
- History of close contact either with poultry (e.g., visited a poultry farm, a household raising poultry, or a bird market) in an H5N1-affected country, or with a known or suspected human case of influenza A (H5N1) within 10 days prior to onset of symptoms.

Appendix 3.
Reference testing guidelines for potential pandemic strains of influenza

State and local laboratories may conduct initial testing on patient specimens for influenza A or potential highly pathogenic strains, if laboratory capacity is available. Due to the spread of avian influenza A (H5N1) in poultry in Asia, laboratories should be on the alert for avian and human H5 viruses. Procedures for diagnosis of human cases of influenza A (H5N1) are provided in Appendix 2. Influenza A viruses other than currently circulating H1 and H3 subtypes should also be considered as potentially pandemic if detected in humans.

•ASL should send specimens to CDC if:
•A sample tested by ASL is positive for H5 or another novel subtype;

Note: *A laboratory should test for influenza A (H5) only if it is able to do so by PCR or has a BSL-3-enhanced facility for influenza A(H5) viral culture.*

or

•A sample from a patient who meets the clinical and epidemiologic criteria for possible infection with a potentially pandemic virus is positive for influenza A by RT-PCR or rapid antigen detection,* is negative for influenza A(H1) and A(H3), and the referring jurisdiction is not equipped to test for specific strains;

or

•The referring jurisdiction is not equipped to test samples for novel influenza viruses by RT-PCR and is requesting testing at CDC.

Shipping procedures for potential pandemic strains of influenza are provided in Appendix 5.

*Because the sensitivity of commercially available rapid diagnostic tests for influenza may not always be optimal, CDC will also accept specimens taken from persons who meet the clinical and epidemiological criteria even if they test negative by influenza rapid diagnostic testing—if PCR assays are not available at the state laboratory.

Appendix 4.
Laboratory biosafety guidelines for handling and processing specimens or isolates of novel influenza strains

Key Messages

- Commercial antigen detection testing for influenza may be conducted under BSL-2 containment conditions if a Class II biological safety cabinet is used.
- Clinical specimens from suspected novel influenza cases may be tested by RT-PCR using standard BSL-2 work practices in a Class II biological safety cabinet for initial processing of patient specimens.
- If a specimen is confirmed positive for influenza A (H5N1) by RT-PCR, additional testing should be performed only under BSL-3 conditions with enhancements. CDC's Influenza Branch should be informed immediately by contacting the CDC Director's Emergency Operations Center (DEOC) at 770-488-7100.
- A detailed description of recommended facilities, practices, and protective equipment for the various laboratory biosafety levels can be found in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)manual at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
- BSL-3 with enhancements and Animal Biosafety Level 3 include: all BSL-3 practices, procedures, and facilities, plus the use of negative-pressure, HEPA-filtered respirators or positive air-purifying respirators, and clothing change and personal showering protocols. Additional practices and/or restrictions may be added as conditions of USDA-APHIS permits. Registration of personnel and facilities with the Select Agent Program is required for work with highly pathogenic avian influenza (HPAI) viruses, which are classified as agricultural select agents.
- ASL will test clinical specimens from suspected novel influenza cases by RT-PCR using standard BSL-2 work practices in a Class II biological safety cabinet. Commercial rapid antigen detection testing may also be conducted under BSL-2 biocontainment conditions.
- Highly pathogenic avian influenza A (H5) and A (H7) viruses are classified as select agents. USDA regulations require that these viruses (as well as exotic low pathogenic avian influenza viruses) be handled under BSL-3 laboratory containment conditions, with enhancements (i.e., controlled-access double-door entry with change room and shower, use of respirators, decontamination of all wastes, and showering of all personnel). Laboratories that work with these viruses must be certified by USDA.
- Laboratories should not perform virus isolation on respiratory specimens from patients who may be infected with an avian influenza virus unless stringent BSL-3 enhanced containment conditions can be met and diagnostic work can be kept separate from studies with other human influenza A viruses (i.e., H1 or H3). Therefore, respiratory virus cultures should not be performed in most clinical laboratories. Cultures for patients suspected of having influenza A (H5N1)infection should be sent only to state laboratories with appropriate BSL-3 with enhancement containment facilities or to CDC.

Appendix 5.
1/4/2006 guidelines for collecting and shipping specimens for influenza diagnostics

Key Messages

- Appropriate specimens for influenza testing vary by type of test.
- Before collecting specimens, review the infection control precautions are described in Supplement 3.

I. RESPIRATORY SPECIMENS [4]

Eight types of respiratory specimens may be collected for viral and/or bacterial diagnostics: 1) nasopharyngeal wash/aspirates, 2) nasopharyngeal swabs, 3) oropharyngeal swabs, 4) broncheoalveolar lavage, 5) trachealaspirate, 6) pleural fluid tap, 7) sputum, and 8) autopsy specimens. Nasopharyngeal wash/aspirates are the specimen of choice for detection of most respiratory viruses and are the preferred specimen type for children aged <2 years. Respiratory specimens for detection of most respiratory pathogens, and influenza in particular, are optimally collected within the first 3 days of the onset of illness. Before collecting specimens, review the infection control precautions in Supplement 4.

A. Collecting specimens from the upper respiratory tract

1.Nasopharyngeal wash/aspirate

- Have the patient sit with head tilted slightly backward.
- Instill 1 ml–1.5 ml of nonbacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2 ml–3ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate nasopharyngeal secretions. Repeat this procedure for the other nostril. •Collect the specimens in sterile vials. Label each specimen container with the patient's ID number and the date collected.
- If shipping domestically, use cold packs to keep the sample at 4°C. If shipping internationally, pack in dry ice (see shipping instructions below).
- 2.Nasopharyngeal or oropharyngeal swabs
- Use only sterile dacron or rayon swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit PCR testing.
- To obtain a nasopharyngeal swab, insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both nostrils.
- To obtain an oropharyngeal swab, swab the posterior pharynx and tonsillar areas, avoiding the tongue.
- Place the swabs immediately into sterile vials containing 2 ml of viral transport media. Break the applicator sticks off near the tip to permit tightening of the cap. Label each specimen container with the patient's ID number and the date the sample was collected.
- If shipping domestically, use cold packs to keep the sample at 4°C. If shipping internationally, pack in dry ice (see shipping instructions below).

[4]All types of respiratory specimens may used in RT-PCR tests. Fresh-frozen unfixed tissue specimens may also be submitted for RT-PCR.

B. Collecting specimens from the lower respiratory tract

1. Broncheoalveolar lavage, tracheal aspirate, or pleural fluid tap

- During bronchoalveolar lavage or tracheal aspirate, use a double-tube system to maximum shielding fromoropharyngeal secretions.
- Centrifuge half of the specimen, and fix the cell pellet in formalin. Place the remaining unspun fluid in sterile vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®. Label each specimen container with the patient's ID number and the date the sample was collected.
- If shipping domestically, use cold packs to keep the sample at 4°C. If shipping internationally, ship fixed cells at room temperature and unfixed cells frozen (see shipping instructions below).

2.Sputum

- Educate the patient about the difference between sputum and oral secretions.
- Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap sputum collection cup or sterile dry container.
- If shipping domestically, use cold packs to keep the sample at 4°C. If shipping internationally, pack in dry ice (see shipping instructions below).

II. BLOOD COMPONENTS

Both acute and convalescent serum specimens should be collected for antibody testing. Collect convalescent serum specimens 2–4 weeks after the onset of illness. To collect serum for antibody testing:

- Collect 5 ml–10 ml of whole blood in a serum separator tube. Allow the blood to clot, centrifuge briefly, and collect all resulting sera in vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®.
- The minimum amount of serum preferred for each test is 200 microliters, which can easily be obtained from 5 ml of whole blood. A minimum of 1 cc of whole blood is needed for testing of pediatric patients. If possible, collect 1 cc in an EDTA tube and in a clotting tube. If only 1cc can be obtained, use a clotting tube.
- Label each specimen container with the patient's ID number and the date the specimen was collected.
- If unfrozen and transported domestically, ship with cold packs to keep the sample at 4°C. If frozen or transported internationally, ship on dry ice.

III. AUTOPSY SPECIMENS

CDC can perform immunohistochemical (IHC) staining for influenza A (H5) viruses on autopsy specimens. Viral antigens maybe focal and sparsely distributed in patients with influenza, and are most frequently detected in respiratory epithelium of large airways. Larger airways (particularly primary and segmental bronchi) have the highest yield for detection of influenza viruses by IHC staining. Collection of the appropriate tissues ensures the best chance of detecting the virus by (IHC) stains.

- If influenza is suspected, a minimum total of 8 blocks or fixed-tissue specimens representing samples from each of the following sites should be obtained and submitted for evaluation:
- Central (hilar) lung with segmental bronchi
- Right and left primary bronchi
- Trachea (proximal and distal)•Representative pulmonary parenchyma from right and left lung

In addition, representative tissues from major organs should be submitted for evaluation. In particular, for patients with suspected myocarditis or encephalitis, specimens should include myocardium (right and left ventricle) and CNS (cerebral cortex, basal ganglia, pons, medulla, and cerebellum). Specimens should be included from any other organ showing significant gross or microscopic pathology. Specimens may be submitted as:

- Fixed, unprocessed tissue in 10% neutral buffered formalin, or
- Tissue blocks containing formalin-fixed, paraffin-embedded specimens, or
- Unstained sections cut at 3 microns placed on charged glass slides (10 slides per specimen)
- Specimens should be sent at room temperature (NOT FROZEN).
- Fresh-frozen unfixed tissue specimens may be submitted for RT-PCR.
- Include a copy of the autopsy report (preliminary, or final if available), and a cover letter outlining a brief clinical history and the submitter's full name, title, complete mailing address, phone, and fax numbers, in the event that CDC pathologists require further information. Referring pathologists may direct specific questions to CDC pathologists. The contact number for the Infectious Disease Pathology Activity is 404-639-3133, or the pathologists can be contacted 24hours a day, 7 days a week through the CDC Emergency Response Hotline at 770-488-7100.

IV. SHIPPING INSTRUCTIONS

- Local health departments, pathologists, or medical examiners should call ASL, who will coordinate with CDC before sending specimens for influenza A reference testing. CDC Hotline staff will notify a member of the Influenza Branch who will contact ADHS to answer questions and provide guidance. In some cases, the ASL may arrange for a clinical laboratory to send samples directly to CDC.
- Specimens should be sent by Priority Overnight Shipping for receipt within 24 hours. Samples (such as fresh-frozen autopsy samples for RT-PCR or other clinical materials) may be frozen at –70 if the package cannot be shipped within a specified time (e.g., if the

specimen is collected on a Friday but cannot be shipped until Monday).

- When sending clinical specimens, include the specimen inventory sheet (see below), include the assigned CDC case ID number, and note "Influenza surveillance" on all materials and specimens sent.
- Include the CDC case ID number on all materials forwarded to CDC. Protocols for standard interstate shipment of etiologic agents should be followed, and are available at http://www.cdc.gov/od/ohs/biosfty/shipregs.htm . All shipments must comply with current DOT/IATA shipping regulations.

V. INFLUENZA SPECIMEN INVENTORY SHEET

CDC CASE ID:

List specimens sent to the CDC		
Select a SOURCE* from the following list for each specimen: Serum (acute), serum (convalescent), NP swab, NP aspirate/wash, bronchoalveolar lavage specimen (BAL), OP swab, tracheal aspirate, or tissue.		
Specimen Type #1: ❑ Clinical Material ❑ Extracted RNA ❑ Virus Isolate	Source*:_____	Collected : __ __ / __ __ / __ __ __ __ (m m d d y y y y) Date Sent __ __ / __ __ / __ __ __ __ (m m d d y y y y)
Specimen Type #2: ❑ Clinical Material ❑ Extracted RNA ❑ Virus Isolate	Source*:_____	Collected : __ __ / __ __ / __ __ __ __ (m m d d y y y y) Date Sent __ __ / __ __ / __ __ __ __ (m m d d y y y y)
Specimen Type #3: ❑ Clinical Material ❑ Extracted RNA ❑ Virus Isolate	Source*:_____	Collected : __ __ / __ __ / __ __ __ __ (m m d d y y y y) Date Sent __ __ / __ __ / __ __ __ __ (m m d d y y y y)
Specimen Type #4: ❑ Clinical Material ❑ Extracted RNA ❑ Virus Isolate	Source*:_____	Collected : __ __ / __ __ / __ __ __ __ (m m d d y y y y) Date Sent __ __ / __ __ / __ __ __ __ (m m d d y y y y)
Specimen Type #5: ❑ Clinical Material ❑ Extracted RNA ❑ Virus Isolate	Source*:_____	Collected : __ __ / __ __ / __ __ __ __ (m m d d y y y y) Date Sent __ __ / __ __ / __ __ __ __ (m m d d y y y y)
Carrier: _____		Tracking _____

Appendix 6.
Rapid diagnostic testing for influenza

The following information in this appendix is designed to assist clinicians and clinical laboratory directors in the use of rapid diagnostic tests during interpandemic influenza seasons. During an influenza pandemic, one or more of these tests may be sensitive and specific enough to be used by clinicians to supplement clinical diagnoses of pandemic influenza. However, clinicians should be reminded that a negative test result might not rule out pandemic influenza and should not affect patient management or infection control decisions.

I. INFORMATION FOR CLINICIANS

A. Background

Rapid diagnostic tests for influenza can help in the diagnosis and management of patients who present with signs and symptoms compatible with influenza. They also are useful for helping to determine whether institutional outbreaks of respiratory disease might be due to influenza. In general, rapid diagnostic testing for influenza should be done when the results will affect a clinical decision.
Rapid diagnostic testing can provide results within 30 minutes.

B. Reliability and interpretation of rapid test results

The reliability of rapid diagnostic tests depends largely on the conditions under which they are used. Understanding some basic considerations can minimize being misled by false-positive or false-negative results.

Median sensitivities of rapid diagnostic tests are generally ~70%–75% when compared with viral culture, but median specificities of rapid diagnostic tests for influenza are approximately 90%–95%. False-positive (and true negative) results are more likely to occur when disease prevalence in the community is low, which is generally at the beginning and end of the influenza season. False-negative (and true positive) results are more likely to occur when disease prevalence is high in the community, which is typically at the height of the influenza season.

C. Minimizing the occurrence of false results

- Use rapid diagnostic tests that have high sensitivity and specificity.
- Collect specimens as early in the illness as possible (within 4–5 days of symptom onset).
- Follow the manufacturer's instructions, including those for handling of specimens.
- Consider sending specimens for viral culture when:
- Community prevalence of influenza is low and the rapid diagnostic test result is positive, *or*
- Disease prevalence is high but the rapid diagnostic test result is negative.
- (Contact your ADHS or your county health department for information about influenza activity.)

D. For further information

Information about influenza is available at www.azdhs.gov/flu or the CDC influenza website (www.cdc.gov/flu) or from the CDC FluInformation Line (800-CDC-INFO [English and Spanish]; 800-243-7889 [TTY]).

For more information about influenza diagnostics, contact:

Arizona State Public Health Laboratory:
250 North 17th Avenue
Phoenix, AZ 85007
Attn: Virology
(602) 542-6134

Additional resources:

- Association of Public Health Laboratories: http://www.aphl.org/Public_Health_Labs/index.cfm
- Weekly U.S. influenza activity reports: http://www.cdc.gov/flu/weekly/fluactivity.htm
- CDC Clinician Outreach and Communication Activity: http://www.bt.cdc.gov/coca/index.asp
- CDC website: http://www.cdc.gov/flu/professionals/labdiagnosis.htm

II. INFORMATION FOR CLINICAL LABORATORY DIRECTORS

A. Background

Rapid diagnostic tests for influenza are screening tests for influenza virus infection; they can provide results within 30minutes. The use of commercial influenza rapid diagnostic tests by laboratories and clinics has increased substantially in recent years. At least ten rapid influenza tests have been approved by the U.S. Food and Drug Administration (FDA) (see Appendix 1).

Rapid tests differ in some important respects. Some can identify influenza A and B viruses and distinguish between them; some can identify influenza A and B viruses but cannot distinguish between them. Some tests are waived from requirements under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). Most tests can be used with a variety of specimen types, but sensitivity and specificity can vary with specimen type. FDA approval is based upon specific specimen types. Rapid tests vary in terms of sensitivity and specificity when compared with viral culture. Product insert information and research publications indicate that median sensitivities are approximately 70%–75% and median specificities are approximately 90%–95%. Specimens to be used with rapid tests generally should be collected as close as possible to the start of symptoms and usually no more than 4–5 days later in adults. In very young children, influenza viruses can be shed for longer periods; therefore, in some instances, testing for a few days after this period may still be useful. Test sensitivity will be greatest in children, who generally have higher viral titers, if the specimen is obtained during the first 2 days of illness, and if the clinician or laboratory has more experience performing the test. The quality of the specimen tested also is critical for test sensitivity.

B. Accuracy depends on disease prevalence

The positive and negative predictive values of rapid tests vary considerably depending on the prevalence of influenza in the community. False-positive (and true negative) influenza test results are more likely to occur when disease prevalence is low, which is generally at the beginning and end of the influenza season. False-negative (and true positive) influenza test results are more likely to occur when disease prevalence is high, which is typically at the height of the influenza season.

1.Clinical considerations when influenza prevalence is low

When disease prevalence is low, the positive-predictive value (PPV) is low and false-positive test results are more likely. By contrast, the negative-predictive value (NPV) is high when disease prevalence is low, and negative results are more likely tobe truly negative (see Graphs 1 and 2).

If flu prevalence is…	and specificity is…	then PPV is…	false-positive rate is…
VERY LOW (2.5%)	POOR (80%)	V POOR (6%–12%)	V. HIGH (88%–94%)
VERY LOW (2.5%)	GOOD (98%)	POOR (39%–56%)	HIGH (44%–61%)
MODERATE (20%)	POOR (80%)	POOR (38%–56%)	HIGH (44%–62%)
MODERATE (20%)	GOOD (98%)	GOOD (86%–93%)	LOW (7%–14%)

Interpretation of positive results should take into account the clinical characteristics of the case-patient. If an important clinical decision is affected by the test result, the rapid test result should be confirmed by another test, such as viral culture or PCR.

2. Clinical considerations when influenza prevalence is high

When disease prevalence is relatively high, the NPV is low and false-negative test results are more likely. By contrast, when disease prevalence is high, the PPV is high and positive results are more likely to be true (see Graph 2).

If flu prevalence is…	and sensitivity is…	then NPV is…	false-negative rate is.
MODERATE (20%)	POOR (50%)	MODERATE (86%–89%)	MODERATE (11%–14%)
MODERATE (20%)	HIGH (90%)	V. GOOD (97%–99%)	V. LOW (2%–3%)
HIGH (40%)	POOR (50%)	MODERATE (70%–75%)	MODERATE (25%–30%)
HIGH (40%)	HIGH (90%)	V. GOOD (93%–94%)	LOW (6%–7%)

Interpretation of negative results should take into account the clinical characteristics of the case-patient. If an important clinical decision is affected by the test result, the rapid test result should be confirmed by another test, such as viral culture or PCR.

C. Selecting tests

Selection of a test should take into consideration several factors, such as the types of specimens that are considered optimal for that test. Also, tests with high sensitivity and specificity will provide better positive and negative predictive values. Information about test characteristics is provided in product inserts and scientific articles and by the manufacturer.

D. Changes in recommended procedures can affect test results

Modification by the user can affect test performances and increase false-positive and/or false-negative rates. Such modifications include using specimens for which the test is not optimized or using swabs that did not come with the rapid test kit (unless recommended).

E. When are rapid diagnostic tests beneficial?

Use of rapid diagnostic tests are beneficial in these situations:

- To test cases during an outbreak of acute respiratory disease to determine if influenza is the cause, *or*
- To test selected patients during the influenza season, *or*
- In the fall or winter, to test selected patients presenting with respiratory illnesses compatible with influenza to help establish whether influenza is present in a specific population and to guide healthcare providers in diagnosing and treating respiratory illnesses.

In general, the exclusive use of rapid tests does not address the public health need for obtaining viral isolates so that influenza virus strain subtyping and characterization can be conducted to monitor antigenic and genetic changes.

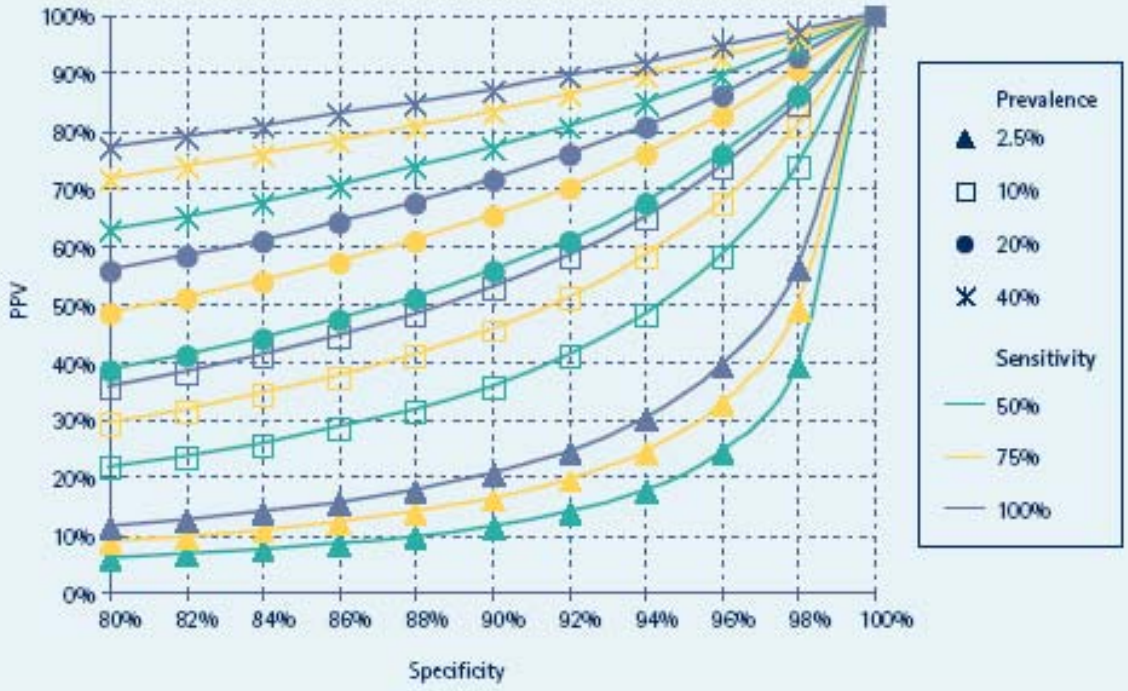
During an influenza pandemic, some rapid diagnostic tests may be able to detect the pandemic strain with adequate sensitivityand specificity. Rapid tests can be used by physicians to supplement clinical diagnoses of pandemic influenza.

Physicians should be reminded that a negative test result might not rule out influenza and should not affect patient management or infection control decisions.

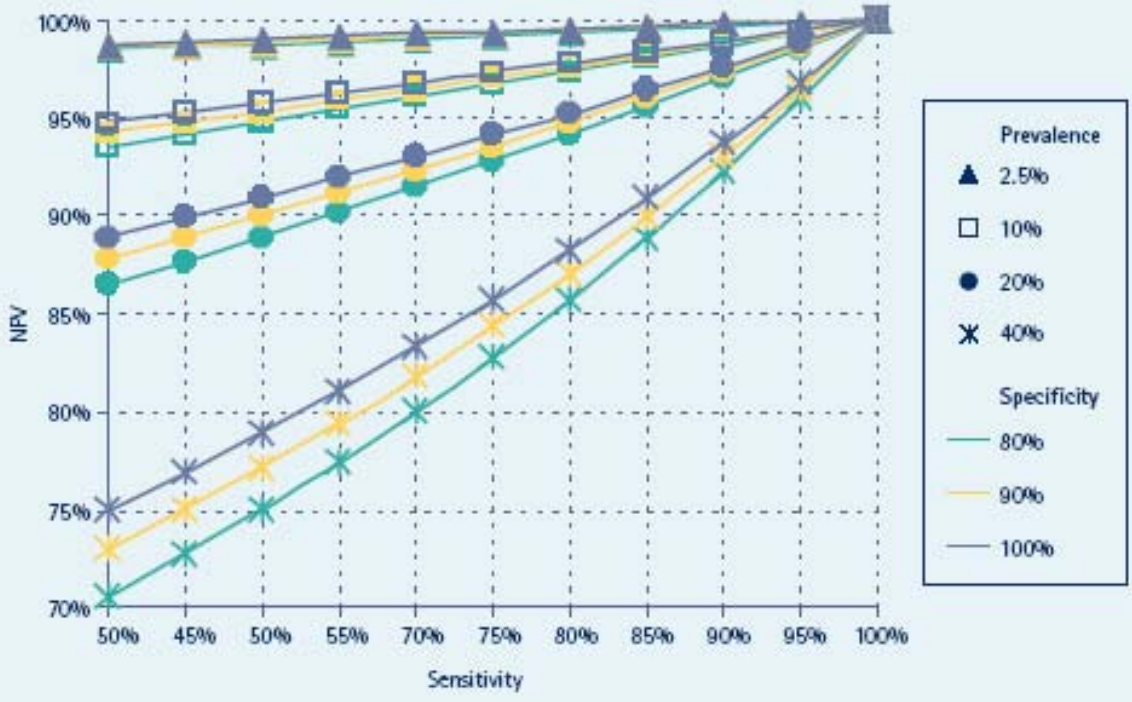
F. For further information

Information on influenza diagnostics is provided on the CDC website at: http://www.cdc.gov/flu/professionals/labdiagnosis.htm .

GRAPH 1: IMPACT OF PREVALENCE, SENSITIVITY AND SPECIFICITY ON POSITIVE PREDICTIVE VALUE



GRAPH 2: IMPACT OF PREVALENCE, SENSITIVITY AND SPECIFICITY ON NEGATIVE PREDICTIVE VALUE



Appendix 7.
Guidelines for medical surveillance of laboratory research personnel working with novel strains of influenza, including avian strains and other strains with pandemic potential

Key Messages

- Laboratory workers should receive training on the appropriate biosafety level for the type of work being performed.
- Before working with avian influenza A viruses, including highly pathogenic strains, laboratory workers should have a baseline serum sample obtained and stored for future reference.
- Workers in laboratories that contain avian influenza A viruses should report any fever or lower respiratory symptoms to their supervisors. Workers should be evaluated for possible exposures, and the clinical features and course of the illness should be closely monitored.
- Laboratory workers who are believed to have had a laboratory exposure to an avian influenza A virus or other highly pathogenic strain should be evaluated, counseled about the risk of transmission to others, and monitored for fever or lower respiratory symptoms as well as for any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, diarrhea.
- ADHS and/or county health departments should be notified promptly of laboratory exposures and illnesses in exposed laboratory workers. Medical surveillance of laboratory personnel can help to ensure that workers who are at risk of occupational exposure to avian influenza viruses or other novel animal or human influenza strains and who develop symptoms of illness receive appropriate medical evaluation and treatment, both for the benefit of their health and to prevent further transmission.

I. PREREQUISITES FOR WORKING WITH NOVEL AVIAN OR HUMAN INFLUENZA VIRUSES

A. Baseline serum samples

Before working with novel avian or human influenza viruses, laboratory workers should have a baseline serum sample obtained and stored for future reference.

B. Influenza vaccine

Laboratories should offer the current inactivated influenza vaccine to laboratory personnel. Its use is especially encouraged for personnel working with avian viruses in BSL-3 enhanced laboratory conditions and for those who may be exposed to these viruses in the field. Immunization might reduce the chance of illness from exposure to human influenza viruses currently circulating in the community that could lead to confusion in monitoring for avian influenza A infection. Vaccines against novel influenza A viruses (e.g., H5N1) are undergoing clinical trials and might be available in the future.

C. Oseltamivir prophylaxis

- It is not necessary to require oseltamivir for laboratory research personnel working with highly pathogenic influenza strains, but encourage it for those doing animal experiments only for the time they are working with animals and especially while working with ferrets.
- When considering oseltamivir prophylaxis, be sure to evaluate appropriate candidates for contraindications, answer their questions, review adverse effects, and explain the benefits.
- Maintain a log of persons on oseltamivir, persons evaluated and not on oseltamivir, doses dispensed, and adverse effects.
- Periodically evaluate and update oseltamivir policies and procedures.

D. Post-exposure prophylaxis

Conditions for use of oseltamivir for post-exposure prophylaxis include a known or suspected laboratory exposure to live avian influenza virus, including highly pathogenic strains, for a person not on oseltamivir. Appropriate healthcare personnel should be available to evaluate immediately and dispense oseltamivir if the exposure occurs during working hours. If exposure occurs after working hours, an exposed laboratory person should present to the Emergency Department and, after evaluation, communicate with ADHS or CDC for recommendations.

II. MANAGEMENT OF INFLUENZA-LIKE ILLNESS IN PERSONNEL WITH POSSIBLE EXPOSURE TO NOVEL AVIAN OR HUMAN INFLUENZA VIRUSES

A. General procedures

- Maintain a daily sign-in/out sheet to record name, date, time in/out, use of oseltamivir, and brief description of job tasks. This record will facilitate retrospective documentation if an illness occurs.
- Workers should report any influenza-like illness and any potential laboratory exposures to the supervisor (see also Supplement 4).

B. Evaluation and treatment

1. During regular working hours

- The affected employee should notify the supervisor. The supervisor should immediately contact the appropriate healthcare personnel and facility contacts (e.g., occupational health, infection control, or designee).
- Upon arrival at the designated clinic, the employee should be placed in a private room for isolation where a health care provider can provide consultation and evaluation.
- The healthcare provider should obtain a respiratory specimen (e.g. nasopharyngeal swab or aspirate) for viral culture. A rapid antigen test[5] with the ability to differentiate between influenza A and B should be used for initial diagnosis, followed by virus isolation.

[5]If laboratory capacity is available; RT-PCR should be used to rule out the suspected pathogen.

- Based on: 1) the rapid test result (if influenza A positive), 2) the status of oseltamivir prophylaxis, and 3) the clinical evaluation, the healthcare provider should determine whether the patient will return to work, be sent home, or be sent to an infectious disease consultant.

2. During working hours when the employee calls from home

- The employee should notify the supervisor. The supervisor should discuss the situation with the appropriate healthcare personnel and determine where and by whom the employee will be evaluated and specimens for viral culture will be obtained.
- The employee may come to an on-site clinic for evaluation or may elect to see a personal physician. If the employee chooses to see a personal physician, the on-site clinician should discuss with the personal physician the likelihood of alaboratory-acquired infection. The personal physician should be asked to collect specimens for antigen detection and viral culture.
- An employee who is not sick enough to be admitted to a hospital should remain at home under the care of a personal physician, pending results from the viral culture. If influenza A (H3N2) or A (H1N1) is identified, the employee should be advised and can resume normal activities as soon as symptoms subside.
- If avian influenza A (e.g., H5, H7, H9) is identified, the family and other contacts should be monitored for illness.[6]
- Local public health officials should be notified about any confirmed avian influenza infections.

3. After working hours

- The employee should notify the supervisor. The supervisor should inform other persons as the situation dictates.
- If the employee is acutely ill with symptoms consistent with influenza, the employee and/or supervisor should contact the appropriate healthcare provider for instructions. The healthcare provider should conduct the initial evaluation and patient management.
- The supervisor should immediately ask the healthcare provider to collect specimens for rapid testing and viral culture.
- The employee should follow the advice of the healthcare provider with regard to further evaluation/treatment.

Appendix 8.
Contact Information and Resources

Contact Information

Arizona State Public Health Laboratory
250 North 17th Avenue
Phoenix, AZ 85007
Attn: Virology
(602) 542-6134

After-hours emergency contact:
Laboratory Manager's on-call pager – (602) 591-8683

Influenza: Resources

ADHS homepage for influenza
http://www.azdhs.gov/flu

ADHS homepage for influenza pandemic preparedness
http://www.azdhs.gov/pandemicflu

CDC home page for influenza
http://www.cdc.gov/flu
http://www.cdc.gov/flu/weekly/fluactivity.htm

U.S. web site for pandemic flu & U.S. Pandemic Flu Plan and Preparedness Planning
http://pandemicflu.gov/

W.H.O. home page for influenza (including avian influenza)
http://www.who.int/csr/disease/influenza/en/

Promed (Program for Monitoring Emerging Diseases, International Society for Infectious)
http://www.promedmail.org

U.S. Food & Drug Administration (FDA)
http://www.fda.gov/cdrh/oivd/tips/rapidflu.html

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 4th ed
http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm